EXHIBIT B20
Part 3

RayBio # ELH-CA125

Test unconcentrated media vs concentrated using Amicon Ultra-15 filter MW cutoff 10,000

Weigh tubes	Volume	~~empty~~ sample reservoir ~~w/ media~~	empty centrifuge tube	media + reservoir
266	8.36mL ~~25.0g~~	10.99g	11.59g	19.17g
338	8.853mL ~~24.9g~~	10.98g	11.53g	19.7g

Spin tubes for 25 min at 4000xg

move the retentate by pipetting into new container

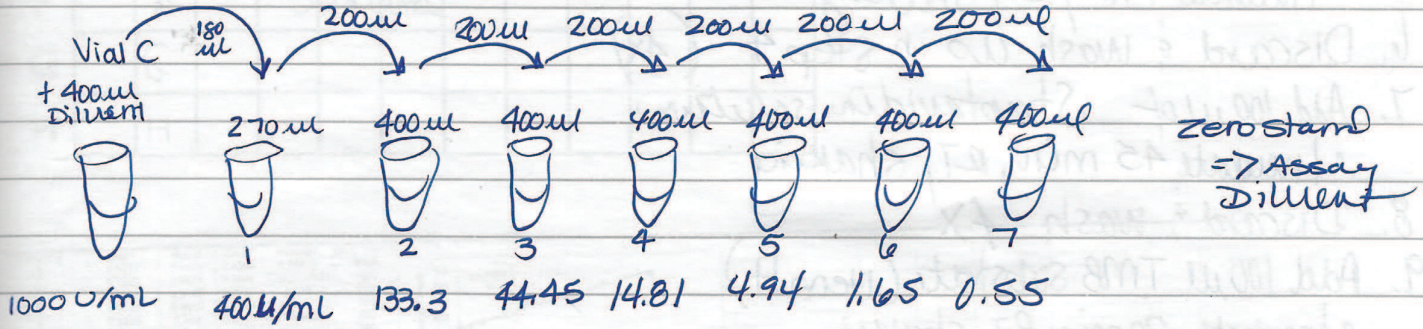
weigh retentate	tube weight	tube + retentate	filtrate
266	1.0025g	1.3514g	19.29g
338	1.0088g	1.7104g	19.4497g

$$\% \text{ retentate recovery} = 100 \times \frac{W_r \times C_r}{W_o \times C_o}$$

$$\% \text{ filtrate recovery} = 100 \times \frac{W_f \times C_f}{W_o \times C_o}$$

% recovery = % retentate recovery + % filtrate recovery

1. All reagents & samples to room temperature
2. Assay diluent (Item E2) should be diluted 5x w/ dd H_2O
   - Stable 1mo. at 4°C
3. Prep. of Standard
   - Spin vial C
   - Add 400 µl 1x Assay Dilvent into vial C = 1000 u/mL
     - mix gently



4. Prepare Wash buffer by diluting 20x
   - Stable 1mo. at 4°C

5. Spin Item F, detection antibody
   - Add 100 µl of 1X Assay Dilvent
     - Stable 5days at 4°C
   - Dilute it ~~80x~~ 800x (use assay diluent) and will be used in assay

6. Spin HRP-Strep. Vial (Item G) & mix
   - Add 15µl to tube w/ 12mL of assay diluent (800x)
     - do not save!

Go to pg 10

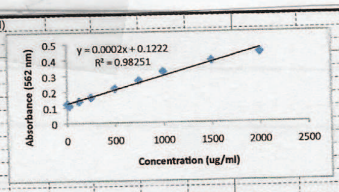
Assay Procedure

1. All samples to RT.
2. Label 8 strip/wells
3. Add 100µl standard / samples
   - Incubate 2.5 hr, gentle shaking, RT
4. Discard solution, wash 4x
   - decant, blot after washes
5. Add 100µl of 1x Antibody mix
   - Incubate 1 hr, RT, shaking
6. Discard & wash as in step 4 (4x)
7. Add 100µl of Streptavidin solution
   - Incubate 45 min, RT, shaking
8. Discard & wash 4x
9. Add 100µl TMB substrate (Item H)
   - Incubate 30 min, RT, shaking
10. Add 50µl STOP solution (Item I) to each well
    - Read at 450nm

1/10/18 Protein levels in media for CA125 Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	A		Stand		I						
B		B		Blank								
C		C		266 orig								
D		D		338 orig								
E		E		266 Conc.								
F		F		338 Conc.								
G		G										
H		H										

Standard	Raw1	Raw2	Raw3	Average	Conc (ug/ml)
A	0.5417	0.4288	0.4595	0.44415	2000
B	0.3952	0.3871	0.3944	0.392233333	1500
C	0.3347	0.3186	0.3157	0.323	1000
D	0.2741	0.2663	0.2583	0.266233333	750
E	0.2216	0.214	0.2153	0.216966667	500
F	0.1669	0.1608	0.1584	0.162033333	250
G	0.1374	0.1406	0.1338	0.137233333	125
H	0.1105	0.1072	0.1072	0.1083	25
I	0.1171	0.1195	0.1258	0.1208	5
Blank	1.0783	1.1058	1.0779	1.09185	0



	Raw1	Raw2	Raw3	Corr 1	Corr2	Corr3	ug/ml 1	ug/ml 2	ug/ml 3	ug/ul	ug/ul	ug/ul	average	ul to use	diluent to u	ug protein	In triplicate ul to use	diluent to use	
266 original	0.8548	0.8586	0.8643				3663	3682	3710.5	3.663	3.682	3.711	3.7	95.0	5.0	350	332.4	17.6	266
338 original	0.952	0.9891	1.0074				4149	4334.5	4426	4.149	4.335	4.426	4.3	81.3	18.7	350	284.7	65.3	338
226 concentrated	4	4	4				19389	19389	19389	19.389	19.389	19.389	19.4	100.0000	0		350.0	0.0	226
338 concentrated	4	4	4				19389	19389	19389	19.389	19.389	19.389	19.4	100.0000	0.0		350.0	0.0	338

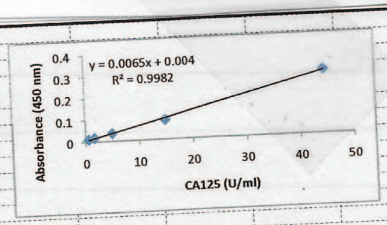
CA125 ELISA - test levels in media

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	1		266 orig								
B		2		338 orig.								
C		3		266 conc								
D		4		338 conc								
E		5		media blank								
F		6										
G		7										
H	Blank											

1/11/18

Test media amounts for CA125 ELISA

Standard (U/ml)	OD1 (450 nr	OD1	Corrected OD1	Corrected OD2	Average
400	2.3856	2.3921	2.31895	2.32545	2.3222
133.3	1.1825	1.1458	1.11585	1.07915	1.0975
44.45	0.3643	0.358	0.29765	0.29135	0.2945
14.81	0.1593	0.1562	0.09265	0.08955	0.0911
4.94	0.1049	0.1009	0.03825	0.03425	0.03625
1.65	0.082	0.0861	0.01535	0.01945	0.0174
0.55	0.0769	0.0776	0.01025	0.01095	0.0106
Blank	0.0661	0.0672	0.06665		



Samples	OD1		OD2		OD3	Corrected OD1	Corrected OD2	Corrected OD3	Corrected for Media OD1	Corrected for Media OD2	Corrected for Media OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
Original 266	0.096	0.0925	0.0939	0.02935	0.02585	0.02725	0.0155	0.012	0.0134	1.769230769	1.230769231	1.446153846	1.482051282	0.271019698
Original 338	0.1179	0.1155	0.1172	0.05125	0.04885	0.05055	0.0374	0.035	0.0367	5.138461538	4.769230769	5.030769231	4.979487179	0.189882139
Concentrated 266	0.0849	0.0799	0.0843	0.01825	0.01325	0.01765	0.0044	-0.0006	0.0038	0.061538462	-0.70769231	-0.03076923	-0.22564103	0.42001221
Concentrated 338	0.2216	0.2312	0.2172	0.15495	0.16455	0.15055	0.1411	0.1507	0.1367	21.09230769	22.56923077	20.41538462	21.35897436	1.101406667
Plain Media	0.0799	0.0885	0.0811	0.01325	0.02185	0.01445	0.01385									

266 = TOV1120 cell

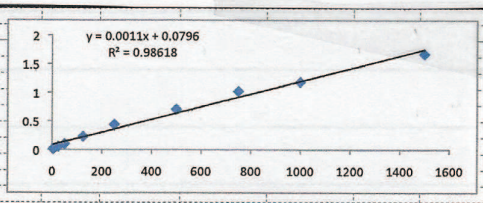
338 = Normal Ov. Epi Cells

Proceed using unconcentrated media

Protein levels for CA125 assay

Re-did standard and original media - media was too concentrated
- diluted media by 50%, remeasured
- Also used 25 ul of the standard and samples

1/12/18	Using 25 ul of standard							
Standard	Raw1	Raw2	Raw3	Corr1	Corr2	Corr3	Average	Conc.(ug/ml)
B1	1.8393	1.7711	1.7805	1.736933333	1.66873333	1.678	1.673433333	1500
C1	1.277	1.2876	1.2926	1.174633333	1.18523333	1.19	1.183366667	1000
D1	1.1027	1.1374	1.1232	1.000333333	1.03503333	1.021	1.018733333	750
E1	0.8031	0.8012	0.8102	0.700733333	0.69883333	0.708	0.702466667	500
A	0.5556	0.527	0.5415	0.453233333	0.42463333	0.439	0.439	250
B	0.336	0.311	0.3407	0.233633333	0.20863333	0.238	0.226866667	125
C	0.2049	0.1908	0.2023	0.102533333	0.08843333	0.1	0.096966667	50
D	0.157	0.1555	0.154	0.054633333	0.05313333	0.052	0.053133333	25
E	0.118	0.1119	0.1112	0.015633333	0.00953333	0.009	0.009183333	5
Blank	0.1036	0.1008	0.1027	0.102366667				0



												ug to use per well = 350	
Samples	Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ml 1	ug/ml 2	ug/ml 3	Avg	x df	ul sample	ul dilutent
279	2.2288	2.2411	2.2644	2.1337	2.146	2.169	1.867363636	1.87854545	1.89973	1.88188	3.76375758	325.5	24.5
281	2.4279	2.366	2.3468	2.3328	2.2709	2.252	2.048363636	1.99209091	1.97464	2.00503	4.01006061	305.5	44.5
297	3.5204	3.4285	3.3964	3.4253	3.3334	3.301	3.041545455	2.958	2.92882	2.97612	5.95224242	205.8	144.2
299	3.3355	3.3229	3.4021	3.2404	3.2278	3.307	2.873454545	2.862	2.934	2.88982	5.77963636	212.0	138.0
333	2.7243	2.5049	2.2334	2.6292	2.4098	2.138	2.317818182	2.11836364	1.87155	2.10258	4.20515152	291.3	58.7
335	2.2042	2.4787	2.3071	2.1091	2.3836	2.212	1.845	2.09454545	1.93855	1.95936	3.91872727	312.6	37.4
351	3.0495	3.0067	2.9638	2.9544	2.9116	2.869	2.613454545	2.57454545	2.53555	2.57452	5.1490303	237.9	112.1
353	2.8432	2.768	3.0064	2.7481	2.6729	2.911	2.425909091	2.35754545	2.57427	2.45258	4.90515152	249.7	100.3
Blank	0.0944	0.0981	0.0928	0.0951									

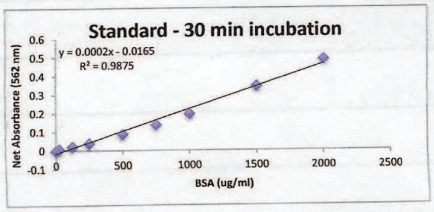
1/12/2018

	Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV112D 72 hr control	279	0.104	0.0885	0.0969	0.03735	0.02185	0.03025	5.13076923	2.74615385	4.03846154	4.58461538	0.77237818
TOV112D 72 hr 1000 ug/ml Talc	281	0.0806	0.0742	0.0838	0.01395	0.00755	0.01715	1.53076923	0.54615385	2.02307692	1.77692308	0.34811411
OV90 72 hr control	297	0.1059	0.1046	0.1321	0.03925	0.03795	0.06545	5.42307692	5.22307692	9.45384615	5.32307692	0.14142136
Ov90 72 hr 1000 ug/ml Talc	299	0.1062	0.0906	0.1316	0.03955	0.02395	0.06495	5.46923077	3.06923077	9.37692308	4.26923077	1.69705627
TOV-21G 72 hr control	333	0.0724	0.0716	0.0942	0.00575	0.00495	0.02755	0.26923077	0.14615385	3.62307692	0.20769231	0.08702853
TOV-21G 72 hr 1000 ug/ml Talc	335	0.0761	0.077	0.0856	0.00945	0.01035	0.01895	0.83846154	0.97692308	2.3	0.90769231	0.09790709
Normal Ov Epithelial 72 hr control	351	0.1003	0.0943	0.1001	0.03365	0.02765	0.03345	4.56153846	3.63846154	4.53076923	4.54615385	0.02175713
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.1106	0.092	0.1331	0.04395	0.02535	0.06645	6.14615385	3.28461538	9.60769231	4.71538462	2.02341325
b		0.0661	0.0672		0.06665							

The other proteins in media may be interfering. Try lysate.

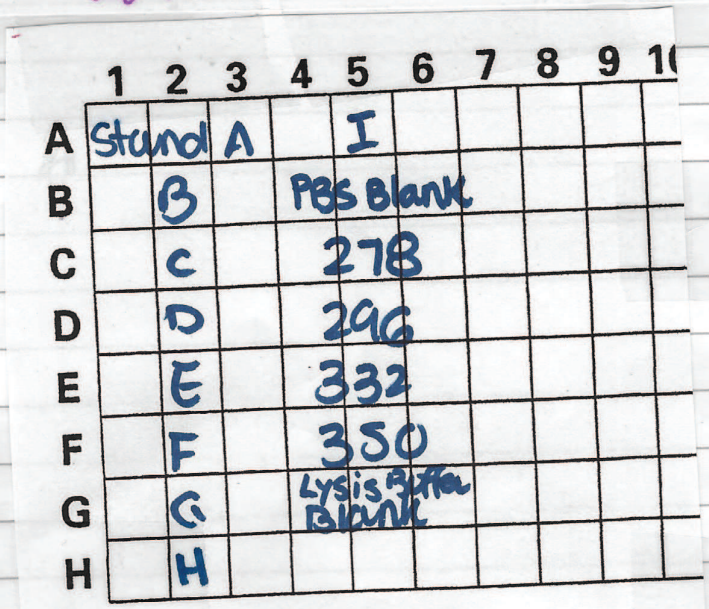
Protein assays in duplicate 1-16-18

Standard ID	Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5869	0.5848	0.5562	0.57596667	0.4874
B	1500	0.4457	0.4211	0.3533	0.4334	0.34483333
C	1000	0.3213	0.2774	0.2593	0.286	0.19743333
D	750	0.2473	0.2199	0.2128	0.22666667	0.1381
E	500	0.181	0.1759	0.1713	0.17606667	0.0875
F	250	0.1328	0.1252	0.1256	0.12786667	0.0393
G	125	0.1105	0.112	0.1116	0.11136667	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.00803333
I	5	0.0845	0.0846	0.0941	0.08773333	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.08856667	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	using 100 ug per well	x 3.5 wells	diluent
TOV112D 72 hr control	279	0.2429	0.2301	0.2326	0.1356	0.1228	0.1253	7.605	6.965	7.09	7.0275	14.23	49.8	300.2
TOV112D 72 hr 1000 ug/ml Talc	281	0.1892	0.1923	0.1878	0.0819	0.085	0.0805	4.92	5.075	4.85	4.94833333	20.21	70.7	279.3
OV90 72 hr control	297	0.3198	0.331	0.3306	0.2125	0.2237	0.2233	11.45	12.01	11.99	12	8.33	29.2	320.8
Ov90 72 hr 1000 ug/ml Talc	299	0.2689	0.2759	0.2784	0.1616	0.1686	0.1711	8.905	9.255	9.38	9.3175	10.73	37.6	312.4
TOV-21G 72 hr control	333	0.2991	0.2968	0.2948	0.1918	0.1895	0.1875	10.415	10.3	10.2	10.305	9.70	34.0	316.0
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1658	0.1616	0.1634	0.0585	0.0543	0.0561	3.75	3.54	3.63	3.64	27.47	96.2	253.8
Normal Ov Epithelial 72 hr control	351	0.2359	0.23	0.23	0.1286	0.1227	0.1227	7.255	6.96	6.96	6.96	14.37	50.3	299.7
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.2432	0.2474	0.2344	0.1359	0.1401	0.1271	7.62	7.83	7.18	7.725	12.94	45.3	304.7
b		0.1097	0.1049		0.1073									

	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	using 100 ug per well	x 3.5 wells	diluent
TOV112D 72 hr unt	278	0.6686	0.6925	0.653	0.56486667	0.58876667	0.54926667	29.0683333	30.2633333	28.2883333	29.2758333	3.42	12.0	338.0
Ov90 72 hr unt 72 hr	296	0.34	0.3407	0.3366	0.23626667	0.23696667	0.23286667	12.6383333	12.6733333	12.4683333	12.5933333	7.94	27.8	322.2
TOV-121G unt	332	0.4392	0.4456	0.4413	0.33546667	0.34186667	0.33756667	17.5983333	17.9183333	17.7033333	17.8108333	5.61	19.7	330.3
Normal Ov Epithelial 72 hr unt	350	0.2281	0.2347	0.2316	0.12436667	0.13096667	0.12786667	7.04333333	7.37333333	7.21833333	7.29583333	13.71	48.0	302.0
b		0.1032	0.104	0.104	0.10373333									



BMS in lysate 1-17-18

	1	2	3	4	5	6	7	8	9	10	11	12
A	279											
B	281											
C	297											
D	299											
E	333											
F	335			BLANK								
G	351											
H	353											

	1	2	3	4	5	6	7	8	9	10	11	12
A											278	
B											278	
C											296	
D											296	
E											332	
F											332	B
G											350	332
H											350	350

1/17/2018 Lysate

Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
279	0.1637	0.1749	0.171	0.0807	0.0919	0.088	11.8	13.5230769	12.9230769	13.2230769	0.42426407
TOV112D 72 hr control											
281	0.1188	0.1175	0.1221	0.0358	0.0345	0.0391	4.89230769	4.69230769	5.4	4.99487179	0.36482413
TOV112D 72 hr 1000 ug/ml Talc											
297	0.146	0.1479	0.1689	0.063	0.0649	0.0859	9.07692308	9.36923077	12.6	9.22307692	0.20669275
OV90 72 hr control											
299	0.1371	0.13	0.1506	0.0541	0.047	0.0676	7.70769231	6.61538462	9.78461538	7.16153846	0.77237818
Ov90 72 hr 1000 ug/ml Talc											
333	0.1374	0.1269	0.1289	0.0544	0.0439	0.0459	7.75384615	6.13846154	6.44615385	6.29230769	0.21757132
TOV-21G 72 hr control											
335	0.1	0.0889	0.0907	0.017	0.0059	0.0077	2	0.29230769	0.56923077	0.43076923	0.19581419
TOV-21G 72 hr 1000 ug/ml Talc											
351	0.1491	0.1518	0.1547	0.0661	0.0688	0.0717	9.55384615	9.96923077	10.4153846	9.76153846	0.29372128
Normal Ov Epithelial 72 hr control											
353	0.1551	0.1362	0.1467	0.0721	0.0532	0.0637	10.4769231	7.56923077	9.18461538	9.83076923	0.91379953
Normal Ov Epithelial 72 hr 1000 ug/ml talc											
b	0.0868	0.0876	0.0746	0.083							

1/18/2018 Lysate

Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
278	0.1998	0.1904		0.0476	0.0382		6.70769231	5.26153846		5.98461538	1.02258519
TOV112D 72 hr unt											
296	0.1978	0.1971	0	0.0456	0.0449		6.4	6.29230769		6.34615385	0.07614996
Ov90 72 hr unt 72 hr											
332	0.1875	0.179	0.199	0.0353	0.0268	0.0468	4.81538462	3.50769231	6.58461538	4.16153846	0.9246781
TOV-121G unt											
350	0.2533	0.2874	0.2328	0.1011	0.1352	0.0806	14.9384615	20.1846154	11.7846154	13.3615385	2.230106
Normal Ov Epithelial 72 hr unt											
b	0.1522										

Results: Lysate protein measurements may be affected by talc.
Repeat protein measurements, have control w/ talc in it.